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Polymer Concentration-Controlled Substrate Specificity in Solvolysis of *p*-Nitrophenyl Alkanoates Catalyzed by 4-(Dialkylamino)pyridine-Functionalized Polymer in Aqueous Methanol Solution

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Abstract

The substrate specificity in solvolysis reactions of *p*-nitrophenyl alkanoates **2** (n=2-18) catalyzed by 4-(dialkylamino)pyridine-functionalized polymer **1** can be controlled by the concentration of **1** in 1:1 (v/v) methanol-water solution at pH 8.0 and 30 °C. Below 1.0×10^{-5} unit mol L^{-1}, macromolecule **1** exhibits substrate specificity for **2** (n=14). As the concentration of **1** increases to 2.5×10^{-5} unit mol L^{-1}, the substrate preference changes from **2** (n=14) to **2** (n=12). The substrate specificity changes again from **2** (n=12) to **2** (n=10) when the concentration of **1** increases further to 7.5×10^{-5} unit mol L^{-1}. The control of substrate specificity by polymer catalyst concentration is believed to be unprecedented for catalysis of ester solvolysis.

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The hydrophobic effects, which are a principal force in determining the structures of proteins and nucleic acids and the binding of substrates to enzymes, play a pivotal role in many chemical phenomena in aqueous solution. Small-molecule amphiphiles are well-known to form aggregates of various morphologies in aqueous solution.[1] Depending on the copolymer composition and the surrounding medium, amphiphilic macromolecules can also form aggregates with multiple morphologies.[2] Extensive studies have shown that the increase of hydrophobic effects of amphiphilic macromolecules, i.e., the increase in ratio of hydrophobic to hydrophilic components and the addition of salting-out agents, leads to changes of aggregate morphology from spheres to rods, and to vesicles in appropriate solvents.[2,3] 4-(Dialkylamino)pyridine-functionalized polymers have been regarded as useful and simple model systems for obtaining a better understanding of the origins of enzymic efficiency and selectivity.[4-6] Recently, we have reported ion-induced substrate specificity in solvolysis of *p*-nitrophenyl alkanoates **2** (n=2-18) catalyzed by polymer **1** containing the 4-(dialkylamino)pyridine functionality and a bis-(trimethylene)disiloxane backbone (Scheme 1).[7] The tris(hydroxymethyl)methylammonium ion as a salting-in ion induces the same substrate specificity for **2** (n=6) in aqueous Tris buffer solution that is obtained with cholesterol

esterase for the same hydrolysis reaction.[8] The addition of salting-out agent NaCl induces a substrate specificity change from **2** (n=14) to **2** (n=12) in 1:1 (v/v) methanol-water solution.[7a] However, control of substrate specificity by the concentration of catalyst has not been reported to date for catalysis of solvolysis reactions.

In this report we describe the first example of substrate specificity controlled by a polymer catalyst concentration in solvolysis reactions of **2** (n=2-18) catalyzed by **1** in 1:1

Scheme 1

(v/v) methanol-water solution. By changing the concentration of **1**, we are able to change the substrate preference in **1**-catalyzed solvolysis of **2** in methanol-water medium. These results are unprecedented for catalysis of ester solvolysis.

The macromolecule **1** is an amphiphilic copolymer containing distinct hydrophobic and hydrophilic regions, and it is synthesized as described before.[6c] The catalytic center of macromolecule **1** is the 4-(dialkylamino)pyridine group and the hydrophobic association of substrate to catalyst in the reaction medium is responsible for the rate enhancements observed in the **1**-catalyzed solvolysis reaction of **2**.[4-7]

We have measured pseudo-first-order rate constants for the **1**-catalyzed solvolysis of **2** (n=2-18) with different concentrations of **1** in 1:1 (v/v) methanol-water solution at pH 8.0 and 30 °C (Figure 1).[9] Without **1**, the solvolysis rate of **2** (n=2-18) is very slow and no substrate specificity is observed in methanol-water solution. In fact, an increase of the alkanoate

Table 1

chain length in **2** causes small decreases in the solvolysis rates. The rates for **1**-catalyzed solvolysis of **2** (n=2-18) increase significantly with increasing concentration of **1**.

Surprisingly, we find that macromolecule **1** demonstrates different substrate preferences as its

Figure 1

concentration increases from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} (Table 1). At 5.0×10^{-6} unit mol L^{-1}, the macromolecule **1** exhibits a preference for **2** (n=14). As the concentration of **1** increases to 1.0×10^{-5} unit mol L^{-1}, the substrate preference is still for **2** (n=14) (Table 1). However, when the concentration of **1** increases to 2.5×10^{-5} unit mol L^{-1}, the substrate specificity changes from **2** (n=14) to **2** (n=12) (Figure 1). For 5.0×10^{-5} unit mol L^{-1}, the substrate specificity also favors **2** (n=12) (Table 1). As the concentration of **1** increases further to 7.5×10^{-5} unit mol L^{-1}, the substrate specificity changes again from **2** (n=12) to **2** (n=10). The latter substrate specificity is also observed for 1.0×10^{-4} unit mol L^{-1} **1** (Table 1). Apparently, the substrate specificity for the **1**-catalyzed solvolysis of **2** is controlled by the concentration of **1** in 1:1 methanol-water solution. Although both enzymes and synthetic catalysts exhibit substrate specificity for the same solvolysis reactions,[5,8,10] we are not aware of any catalytic systems that show substrate specificity controlled by the concentration of catalyst for catalysis of ester solvolysis.

Furthermore, we find that the solutions of **1** show appreciable turbidity when the concentration of **1** is increased beyond 1.0×10^{-4} unit mol L^{-1} in 1:1 methanol-water solutions. These results suggest that changes of the aggregate morphology of **1** from spheres to rods, and to vesicles apparently accompany the increases of concentration of **1** from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} in 1:1 methanol-water solution.[3] A phenomenon well-known for small-molecule amphiphiles is that increases of their concentrations are accompanied by the transition from spherical micelles to micellar rods and vesicles in solution.[11] Recently,

changes of aggregate morphology of polystyrene-*b*-poly-2-vinylpyridine copolymers from spheres to rods, and to vesicles have also been observed with increasing polymer concentration.[12] The continual changes in aggregate morphology are governed solely by the polymer concentration in the solutions.[12] The stretching of hydrophobic chains of macromolecular amphiphiles is known to be greatest when they are located within spherical aggregates, and stretching decreases as the aggregate morphology changes from spheres to rods and decreases further as vesicles are formed.[3] Thus, spherical aggregates tend to provide the strongest hydrophobic binding to lipophilic substrates among the common aggregate morphologies in the reaction medium.

We have made an attempt to compare the decreases of alkanoate chain length (D_L) of **2** associated with substrate specificity changes from **2** (n=14) to **2** (n=12) and **2** (n=10) with the

Table 2

decreases of stretching of hydrophobic chains (D_S) of amphiphilic macromolecules associated with aggregate morphology changes from spheres to rods and vesicles (Table 2). Interestingly, the values of D_L and D_S are almost the same for both processes. The 3% difference between values of D_L and D_S for the change from **2** (n=14) to **2** (n=12) and the change from spheres to rods seems to be reasonable when probable measurement error in the studies of morphological structures by transmission electron microscopy is taken into consideration.[3] These results suggest that a parallel and equivalent decrease in hydrophobic effects is involved in both processes. Therefore, the substrate specificity changes that accompany an increase in the concentration of **1** may be caused by an energetically favorable matching of hydrophobicities of substrate **2** and aggregates of **1** leading to enhanced stabilization of **1•2**

complexes. We suggest that the substrate specificity change from **2** (n=14) to **2** (n=12) may result from a change of aggregate morphology of **1** from spheres to rods leading to decreased hydrophobic binding and increased access of **1•2** complexes to the nucleophilic medium that is optimum for **2** (n=12), whereas the specificity change from **2** (n=12) to **2** (n=10) may be attributed to further change of aggregate morphology of **1** from rods to vesicles leading to further decreased hydrophobic binding compensated by more rapid turn over that is optimum for **2** (n=10). The matching of hydrophobicities of substrate and catalyst is believed to be responsible for the molecular discrimination described by the term hydrophobic interactions at active sites of enzymes and catalysts in controlling substrate specificity for biological and chemical catalysis. Moreover, we find that increasing the concentration of **1** has a parallel effect on the substrate specificity, as does increasing the concentrations of NaCl in 1:1 (v/v) methanol-water solution,[7a] which is consistent with the notion that the hydrophobic effect is at work controlling the substrate specificity in the **1**-catalyzed solvolysis of **2** (n=2-18).

These results demonstrate that the substrate specificity of catalysis of ester solvolysis can be controlled by the concentration of polymer catalyst in the reaction medium, and provide a new approach to the control of chemical reactivity that can be useful in understanding the fundamental basis of controlling substrate specificity at the molecular level in biological and chemical catalysis. The demonstration of polymer concentration-controlled substrate specificity establishes this system as a potential guide to synthetic receptors which can mimic the biological systems in terms of molecular recognition and selective interactions with hydrophobic drugs.

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(9) Kinetic measurements: The cuvette was filled with 2.5 mL of a fresh solution containing catalyst in 1:1 (v/v) methanol-aqueous buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$, pH 8.0) solution and the solution was equilibrated for 10 minutes at 30 °C in the thermostated cell compartment of a Hewlett-Packard Model 8450 spectrophotometer. A stock solution (5 μL) of *p*-nitrophenyl alkanoates (2.5×10^{-2} M) in dioxane was added by microsyringe. The reaction mixture was quickly mixed by shaking and the absorbance at 400 nm was recorded as a function of time. The reactions were performed for 4-5 half-lives and the pseudo-first-order rate constants (k_{obsd}) were obtained as slopes of plots of $\mathrm{Ln}[A_\infty/(A_\infty - A_t)]$ vs time, where A_∞ and A_t are the absorbance at infinite time and time t, respectively. The first-order rate constants (k_{obsd}) represent the average of three runs and experimental error is less than 5%.

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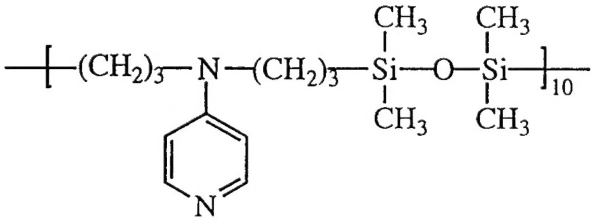
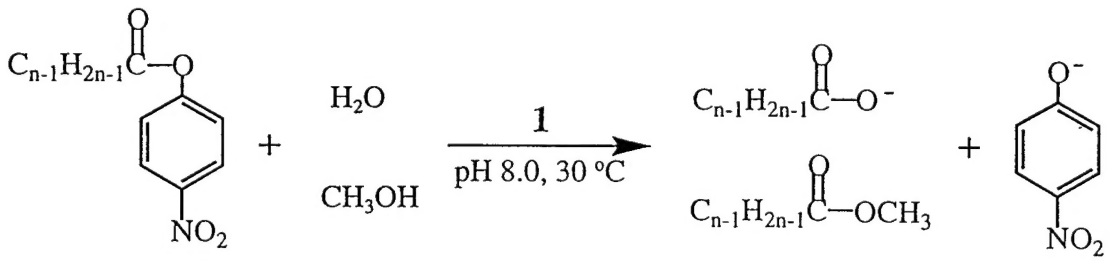
Legend to Figures:

Figure 1. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoates **2** (n=2-18, 5.0×10^{-5} M) catalyzed by **1** as a function of alkanoate chain length (n) in 1:1 (v/v) methanol-aqueous buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$, pH 8.0) solution at 30 °C: •, 7.5×10^{-5} unit mol L^{-1} **1**; ■, 2.5×10^{-5} unit mol L^{-1} **1**; ▲, 5.0×10^{-6} unit mol L^{-1} **1**.



1



2

(n = 2, 4, 6, 8, 10, 12, 14, 16, 18)

Scheme 1

Figure 1

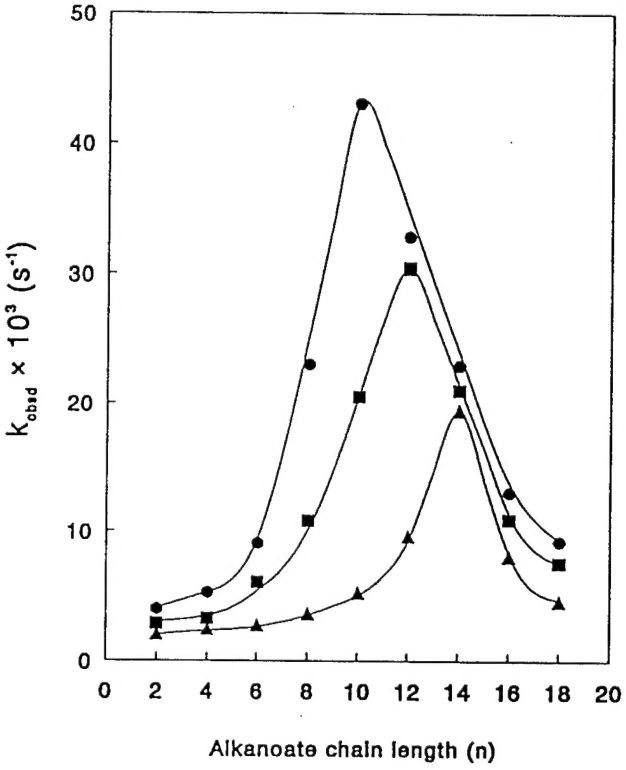

50
40
30
20
10
0
$k_{obsd} \times 10^3 (s^{-1})$
0 2 4 6 8 10 12 14 16 18 20
Alkanoate chain length (n)

Table 1. Summary of the Effect of Concentration of **1** on the Substrate Specificity of **1**-Catalyzed Solvolysis of **2** (n=2-18) in Aqueous Methanol Solution at 30 °C.[a]

Concentration of **1** (unit mol L^{-1})[b]	Substrate specificity
5.0×10^{-6}	**2** (n=14)
1.0×10^{-5}	**2** (n=14)
2.5×10^{-5}	**2** (n=12)
5.0×10^{-5}	**2** (n=12)
7.5×10^{-5}	**2** (n=10)
1.0×10^{-4}	**2** (n=10)

[a]**2**, 5.0×10^{-5} M. [b]In 1:1 (v/v) methanol-aqueous buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$, pH 8.0).

Table 2. The Decrease of Length of Alkanoate Chain (D_L) of **2** Associated with Substrate Specificity Changes from **2** (n=14) to **2** (n=12) and **2** (n=10) and the Decrease of Stretching of Hydrophobic Chains (D_S) of Amphiphilic Macromolecules Associated with Aggregate Morphology Changes from Sphere to Rod and Vesicle.[a]

Change of substrate specificity	D_L[b]	Change of aggregate morphology	D_S[c]
2 (n=14) → **2** (n=14)	0%	Sphere → Sphere	0%
2 (n=14) → **2** (n=12)	14%	Sphere → Rod	11%
2 (n=14) → **2** (n=10)	29%	Sphere → Vesicle	29%

[a]Stretching of hydrophobic chains in aggregate mophology (ratio of the core radius of aggregate to the hydrophobic chain end-to-end distance in the unperturbed state): 1.40 for spheres, 1.25 for rods and 1.00 for vesicles (see Ref. 3a,c). [b]$D_L = (L_{2(n=14)} - L_{2(n=12 \text{ or } 10)})/L_{2(n=14)}$, where L is the length of alkanoate chains of substrates. [c]$D_S = (S_{sphere} - S_{rod \text{ or } vesicle})/S_{sphere}$, where S is the stretching of hydrophobic chains of aggregate morphology.